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Novel fluorescent probe for the selective detection of organophosphorous nerve agents through a cascade reaction from oxime to nitrile via isoxazole

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ABSTRACT

A novel fluorescent probe based on fluoresceinyloxime was developed for detection of such organophosphorous compounds as nerve agents. The probe underwent an abnormal cascade reaction from oxime to nitrile via isoxazole in aqueous solvent and displayed a highly selective fluorescence response to diethylchlorophosphate (DCP) with the detection limit of 10 nM DCP in HEPES buffer.

1. Introduction

Organophosphorous compounds (OPs) are essential ingredients in agricultural pesticides and herbicides, both of which play critical roles in agricultural industry.[1] However, the extensive use of these compounds around the world has caused the unwanted poisoning of thousands of humans. OPs, such as sarin, soman, and tabun have also been used as a G-type of nerve agents in chemical warfare (Fig. 1).[2]

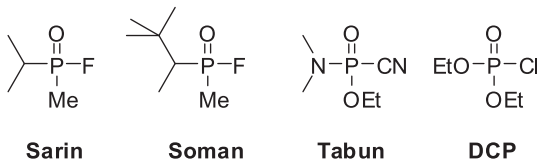

Fig. 1. Chemical warfare agents and their mimic.

Their primary toxicity comes from the inhibition of acetylcholine esterases and inhalation of the OPs can be lethal within minutes by blocking the enzymes. As a consequence, there is a growing interest to develop a sensitive probe for the detection of OPs.[3–5] Recently, Rebek et al. developed a series of probes for OPs by integrating an oxime functional group into salicylaldehyde derivatives; they then observed important but relatively a weak fluorescence enhancement from the oxime-to-isoxazole transformation in the presence of OPs.[6] It is of practical importance to develop a probe capable of naked eye detection by inducing distinct changes both in color and fluorescence in the presence of OPs. Herein we report a fluoresceinyloxime-based probe, which displays both colorimetric and strong fluorescence turn-on changes from the novel mechanism of oxime-to-nitrile conversion in the presence of nerve agent mimics.

2. Materials and methods

2.1. General

All fluorescence and UV–vis absorption spectra were recorded with 10 μM of probe in FP 6500 fluorescence spectrometer and HP 8453 absorption spectrometer, respectively. Both ^{1}H and ^{13}C spectra were recorded at 300 MHz and 75 MHz NMR spectroscopy, respectively. Mass spectra were recorded on G6401A MS-spectrometer. All experiments were carried out with commercially available reagents and solvents, and used without further purification, unless otherwise stated.

2.2. Preparation of fluorescein oxime (1)

First, fluorescein monoaldehyde was prepared according to the previously reported procedure.[7] Fluorescein (3.0 g, 9.0 mmol), 15 g

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NaOH solution (50%) and 5 mL MeOH were placed in a 100 mL flask. Then 8 mL $CHCl_3$ was added dropwisely while the reaction temperature was maintained at 55 °C. The reaction mixture was stirred overnight and then the solvent was removed under the reduced pressure. The residue was purified by column chromatography using CH_2Cl_2/MeOH (v/v 10:1, R_f=0.40) to afford the fluorescein monoaldehyde as a light yellow solid (848 mg, yield 26%).

Next, fluorescein monoaldehyde (720 mg, 2.00 mmol) and hydroxylammonium chloride (206 mg, 3.0 mmol) were dissolved in 10 mL EtOH. The reaction mixture was stirred at room temperature overnight and then the solvent was removed under the reduced pressure. The residue was purified by column chromatography using $CH_3CO_2C_2H_5/CH_2Cl_2$ (v/v 1:7, R_f=0.40) to afford probe **1** as a red solid (402 mg, yield 55%). Mp 267–269 °C, IR (KBr, cm^{-1}): 3250 (br), 1730 (s), 1635 (s), 1582 (s). ^{1}H NMR (300 MHz, DMSO-d_6): 11.91 (s, 1H), 11.10 (s, 1H), 10.18 (s, 1H), 8.86 (s, 1H), 8.01 (d, ^3J=6.6 Hz, 1H), 7.85–7.68 (m, 2H), 7.30 (d, ^3J=6.6 Hz, 1H), 6.87 (s, 1H), 6.74–6.56 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 169.1, 160.0, 159.1, 152.7, 151.8, 149.5, 145.9, 136.1, 130.7, 130.3, 129.4, 126.2, 125.1, 124.5, 113.6, 113.1, 110.4, 109.8, 105.5, 103.1, 83.1. FABMS m/z obsd 376.0821 ([M+H]$^+$, calcd 376.0821 for $C_{21}H_{14}NO_6$).

2.3. Preparation of fluorescein isoxazole (2)

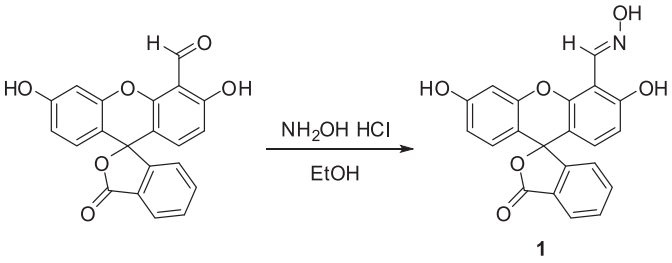
Compound **2** could be prepared by activation of the oxime (**1**) either with diethylchlorophosphate (DCP) or 4-toluenesulfonyl chloride (TsCl). Due to the easy handling process, we herein described the synthetic procedure using TsCl. Compound **1** (186 mg, 0.5 mmol), TsCl (146 mg, 0.75 mmol) and triethylamine (TEA, 68.5 μL, 1.00 mmol) were dissolved in 2 mL CH_3CN. The reaction mixture was stirred at room temperature for 3 h and then the solvent was removed under the reduced pressure. The residue was purified by column chromatography using $CH_3CO_2C_2H_5/CH_2Cl_2$ (v/v 1:25, R_f=0.40) to afford compound **2** as a yellow solid (62 mg, yield 35%). Mp 209–211 °C, IR (KBr, cm^{-1}): 3367 (m), 3082 (w), 1718 (s), 1614 (s), 1447 (s). ^{1}H NMR (300 MHz, DMSO-d_6): 10.33 (s, 1H), 9.64 (s, 1H), 8.05 (d, ^3J=7.2 Hz, 1H), 7.86–7.70 (m, 2H), 7.51 (d, ^3J=8.7 Hz, 1H), 7.33 (d, ^3J=7.2 Hz, 1H), 7.03 (d, ^3J=8.7 Hz, 1H), 6.84 (s, 1H), 6.75–6.58 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 169.0, 163.8, 160.1, 153.2, 151.2, 145.2, 136.3, 131.2, 130.8, 129.8, 126.1, 125.3, 124.6, 114.2, 113.3, 111.6, 109.7, 106.6, 102.8, 82.2. FABMS m/z obsd 358.0719 ([M+H]$^+$, calcd 358.0715 for $C_{21}H_{12}NO_5$).

2.4. Preparation of fluorescein nitrile (3)

Compound **2** (35 mg, 0.10 mmol) was dissolved in 2.2 mL DMSO/water (v/v 2:20). The mixture was stirred at room temperature for 8 h and then extracted with ethyl acetate (EtOAc, 30 mL). The solvent was removed under the reduced pressure. The residue was purified by column chromatography using MeOH/CH_2Cl_2 (v/v 1:20, R_f=0.35) to afford **3** as a red solid. IR (KBr, cm^{-1}): 3250 (br), 2914 (m), 2232 (m), 1736 (s), 1590 (s). ^{1}H NMR (300 MHz, DMSO-d_6): 11.89 (br, 1H), 10.31 (s, 1H), 8.20(d, ^3J=7.2 Hz, 1H), 7.86–7.67 (m, 2H), 7.34 (d, ^3J=7.2 Hz, 1H), 6.89 (d, ^3J=9.0 Hz, 1H), 6.74 (d, ^3J=9.0 Hz, 1H), 6.73 (s, 1H), 6.61 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 169.1, 167.4, 160.9, 153.7, 151.7, 135.8, 133.6, 133.5, 131.9, 130.6, 129.6, 129.1, 127.1, 125.5, 124.8, 115.2, 114.4, 110.0, 107.2, 102.7, 88.2. FABMS m/z obsd 358.0716 ([M+H]$^+$, calcd 358.0715 for $C_{21}H_{12}NO_5$).

3. Result and discussion

Previously we reported an *ortho*-hydroxy functionalized aromatic aldehyde was useful for the detection of toxic- and biochemicals, such as cyanide[8] and thiols.[9] For the fluorescence detection of nerve agents, we grafted the structural motif on the fluorescein, and prepared a fluoresceinyloxime compound by treating hydroxylamine on fluorescein monoaldehyde according to the modified literature procedure (Scheme 1).[10] The probe (**1**) was in good agreement with the reported spectral values.



Scheme 1. Synthesis of **1**.

3.1. Monitoring the cascade reaction by UV–vis spectra

We monitored the expected cyclization reaction (oxime **1** to isoxazole **2**) using UV–vis spectroscopy in the presence of diethylchlorophosphate (DCP, 10 mM), a model compound of nerve agents. With the addition of DCP, we initially observed that the maximum peak of **1** at 496 nm was hypochromically decreased with a clear isosbestic point at 460 nm due to the plausible transformation of an electron-donating phenol group to a relatively less electron-donating isoxazole group (Fig. 2A). From the UV–vis kinetics, we could obtain the initial rate constant of k_1 4.6×10^{-3} M^{-1} s^{-1} at 25 °C through the second order rate kinetics analysis at 420 nm. However, we noticed that the reaction was not complete and the UV–vis spectra was very slowly being converted into another form in aqueous condition. The intermediate peak of **2** at 420 nm was bathochromically shifted to 490 nm with another

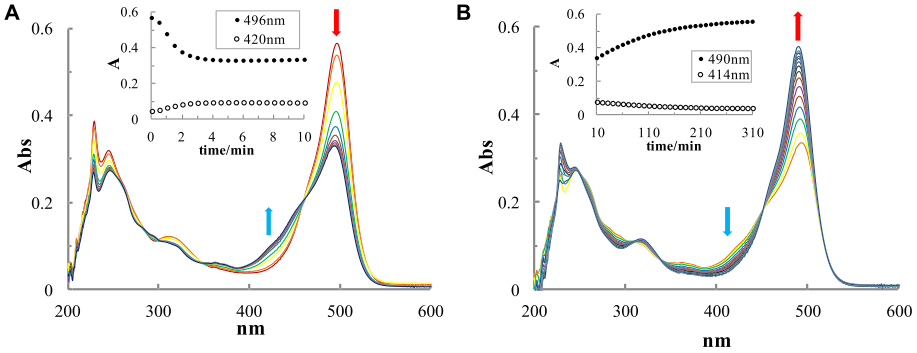


Fig. 2. Time-dependent UV–vis spectral changes of probe 1 (10 μM) with the addition of DCP (10 mM) for 0–10 min (A) and the second spectral changes for 10–240 min (B) in HEPES buffer (0.10 M, pH 7.4). Inset: their kinetics.